Ultramicrostructural Study of Intravenous Fat Emulsion Using a New Fixation Method (II) A Comparative Study on the Effect of Fat Emulsion to the Spleen Between Short Time Infusion of Intravenous Fat Emulsion and the One-Pack Infusion Method

AUTHOR(S):

MIKI, KIICHIRO

Arch Jpn Chir 52(1), 53～66, Jan., 1983

Ultramicrostructural Study of Intravenous Fat Emulsion Using A New Fixation Method

(II) A Comparative Study on the Effect of Fat Emulsion to the Spleen Between Short Time Infusion of Intravenous Fat Emulsion and the One-Pack Infusion Method

KIICHIRO MIKI

Second Department of Surgery, Faculty of Medicine, Kyoto University
(Director: Prof. Dr. YORINORI HIKASA)
Received for Publication, Nov. 15, 1982.

Introduction

It is known that the infused fat emulsion is digested in the reticuloendothelial system (RES). Numerous phagocytes are present in the spleen as a lymph node. As little thought has been given to the important role of the spleen, total splenectomy is generally performed in trauma. Severe overwhelming post-splenectomy syndrome[15,18,20,27,28,32] has become a common problem especially in children. There have been reports concerning the preservation of the healthy part of the injured spleen even in adults[3,25,29,30,31]. However, this attention to adult cases only is recent and may be due to the recognition of increased chance of infection in splenectomised patients. Thus in adults, efforts to preserve healthy part of the injured spleen is now being performed.

The author was prompted to examine spleens which underwent infusion of the fat emulsion, because of several reports of lipidosis or necrosis following the infusion, which may be considered as a drug induced splenectomy. From the viewpoint of ultrastructural morphology the spleen infused with fat emulsion, was studied as follows.

Materials and Methods

Experiment I. Effects of the Fat Emulsion on Morphological Changes in Animals

Twenty four Wister male adult rats were used (the body weight averaged 200 g).

(A) Under intraperitoneal anesthesia with Nembutal, the jugular vein was cut and a fine polyethylene tube specially made was cannulated. Then, the intravenous fat emulsion (10% Intralipid®) 1 ml/kg was infused through the cannulated tube for one hour. As a control group, two additional rats each underwent the same manuever without the fat emulsion.

Then, the animal was laparatomized and the left cavity of the chest was opened. As soon

Key words: Intravenous fat emulsion, Malachite green, Spleen, Infusion fixation, Reticuloendothelial System.
索引語：静注用脂肪乳剤，マラカイトグリーン，網内系，脾臓，灌流固定.
Present adress: Second Department of Surgery, Faculty of Medicine, Kyoto University 54, Shogoin-Kawaracho, Sakyoku, Kyoto 606, Japan.

as the chest was opened, the thoracic aorta was exposed, cut and a tube was cannulated toward the abdominal aorta. The tip of the cannulation tube was inserted near the celiac trunk through this route. The proximal side of the aorta was ligated. Saline solution was infused slowly, the jugular route was used also as a draining channel. Then, the inferior vena cava and the abdominal aorta just under the renal arteries were ligated (Fig. 1).

After washing out the blood circulating in the intraabdominal organs for several minutes, pre-fixative was perfused through thoracic aorta route at 150 cmH_2O. Two kinds of pre-fixatives were used: 2% of glutaraldehyde solution (phosphate buffer pH 7.25), and 2% glutaraldehyde plus 0.1% malachite green solution.

Perfusion time was determined by the hardness of the liver, and the average perfusion time was about one hour. After the perfusion fixation, the spleen was resected and directly prepared in small samples (2×2 mm, thickness about 2 mm) and immersed directly in 1% osmium tetroxide solution. Each sample was separated into two groups. One was prepared for transmission electron microscopy (TEM), the other was prepared for cryofracture observation by scanning electron microscopy (SEM). At the same time Hematoxylin-Eosin (HE) and Sudan III staining for light microscopy of the samples were prepared.

(B) In this group the tip of the cannula in the jugular vein was positioned near the right auricle. The proximal side of the cannulated tube was passed through subcutaneous tissue and it exited at the back near the neck; through this cannula infusion was done. The menu of the infusate was as follows: 2 ml of Intralipid, 5 ml of 12% amino acids, 10 ml of 50% glucose solution and 13 ml of lactated Ringer solution. These mixed solution was infused for 24 hours with an infusion pump. After 24 hours the infused rats were anesthetized again and autopsied. The same preparation for SEM and TEM were performed as mentioned above.

Experiment II. Clinical Studies of Infused Fat Emulsion in the Spleen

Five splenectomized cases were examined as follows:

Case 1; K.U. 22 y.o., female. Total gastrectomy for gastric carcinoma with splenectomy was performed. She did not receive an infusion of the intravenous fat emulsion before operation as a control.

Case 2; Y.T. 32 y.o. female with clinical idiopathic thrombocytopenic purpura (histopathological examination did not confirm it). She received an 500-ml infusion of 10% intravenous fat emulsion for 2 hours just before the operation.

Case 3; K.Y. 76 y.o. female with cystoadenocarcinoma of the body of the pancreas. Distal pancreatectomy with splenectomy was performed. Before the operation the same procedure of Intralipid infusion as in Case 2 was performed.

Case 4; G.M. 76 y.o. male. Intestinal obstruction occurred several weeks after he had a radical operation for rectal carcinoma. During the operation for ileus, the spleen was damaged and extirpated. He had been given total parenteral nutrition (TPN) with one-pack method after being operated on for rectal cancer.

Case 5; T.S. 36 y.o. Splenectomy was performed for portal hypertension. He was given TPN with one-pack method before the operation. (One-pack method is the way to infuse a daily menu

of infusates which were mixed in one soft bag including fat emulsion. Then, the fat emulsion is diluted and infused for 24 hours.)

Immediately after the spleen was extirpated, the splenic artery and vein were quickly exposed. At first, the two major branches of the splenic artery were prepared, which supplied the upper and lower portions. After the cannulation into these two major branches, the infusion of saline with heparin (3000 unit/500 ml) was begun at the pressure commensurated with the patient's average preoperative blood pressure; another cannulation was performed into the splenic vein and the draining venous pressure was maintained at 15 cmH_2O. This maneuver was performed for 10 min in order not to wash out totally blood cells which were necessary for the subsequent observation (Fig. 2).

After the washing perfusion, the perfusion fixation of the spleen was performed. Through one branch of the splenic artery, 2% glutaraldehyde plus 0.1% malachite green solution was simultaneously perfused and through the other branch perfusion fixation was done with only 2% glutaraldehyde. The perfusion fixation was performed for about half an hour. At this time the hardness of the fixed spleen was determined. The spleen was then prepared for TEM and SEM as mentioned previously for animals.

Results

1. Results in the Experimental Studies of Animals

(A) On Sudan III staining, large sudanophilic globules were observed in almost all of the macrophages in the spleen. At high magnification (×1000) these globules appeared to consist of small particles; on HE staining they appeared as vacuoles (Figs. 3 and 4).

By TEM and SEM observations of the splenic samples which underwent perfusion fixation with glutaraldehyde plus malachite green, these globules formed a body of electron dense particles. The globules were very large, often as large or larger than the nuclei of macrophages. Moreover, free particles and small globules were observed in the cytoplasm of the phagocytes. By SEM observation cryofractured samples also showed large globules which were filled with particles. These particles were round with smooth surfaces (Figs. 5, 6, 7 and 10).

On the other hand, in the spleen which underwent perfusion fixation with glutaraldehyde alone, no globules with particles were observed by TEM or SEM observations. By TEM observation, only large vacuoles were observed. In the vacuoles there were no particles and only a matrix-like structure was noted. By SEM observation various overlapped membraneous structures were observed in the vacuoles (Figs. 9 and 10).

In the control group, which did not undergo infusion of fat emulsion, these globules or vacuoles were not observed ultrastructurally. And only sudanophilic material was not observed by light microscopy in either perfusion fixations.

Moreover, intravenous fat pigments were already observed in animals autopsied 24 hours after the single infusion.

(B) Very few small groups of macrophages were found to include sudanophilic materials. However, intravenous fat pigments were seen in almost all of the macrophages in the spleen.

Fig. 1. Photograph of perfusion fixation of a rat abdominal viscera. The infused area is stained with malachite green.

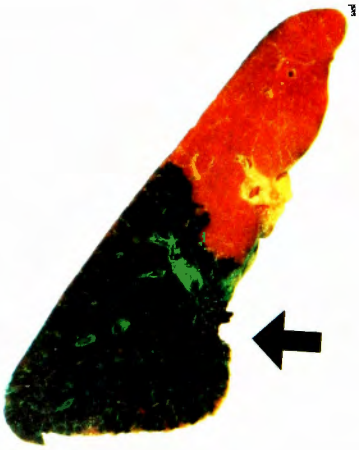

Fig. 2. Photograph of the human spleen which had perfusion fixation with malachite green (lower half, arrowed) and usual perfusion fixation (upper half). Both perfusion fixations were done simultaneously.

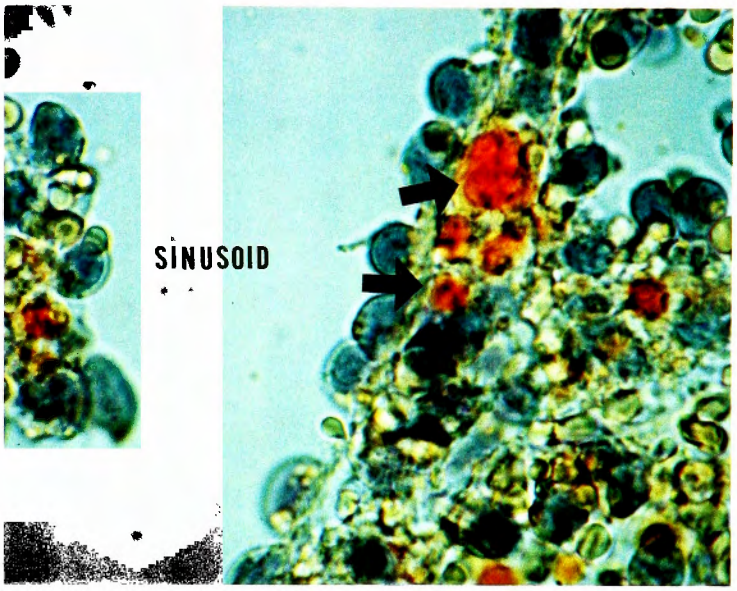

Fig. 3. Sudan III stain of a rat spleen. Vacuoles in the phagocytes were found to be fat material (arrows).

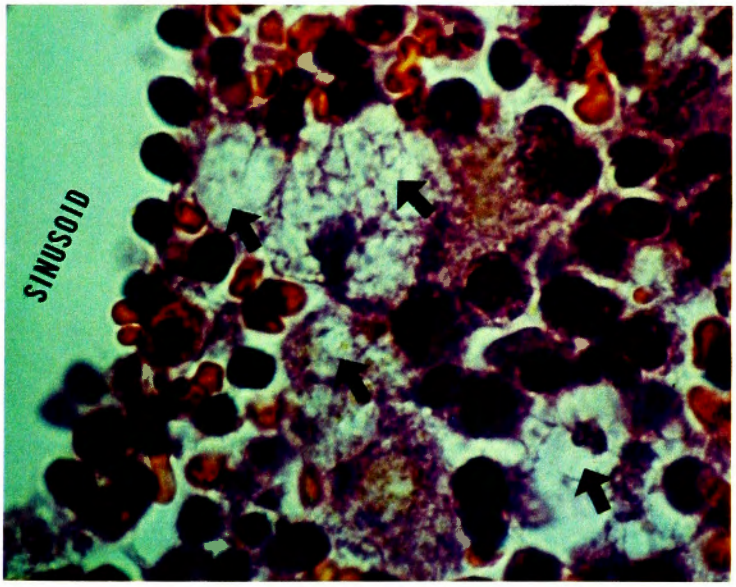

Fig. 4. H E stain of same rat spleen as in Fig. 3 (Sudan stain). Vacuolated macrophages (arrows) are characteristic.

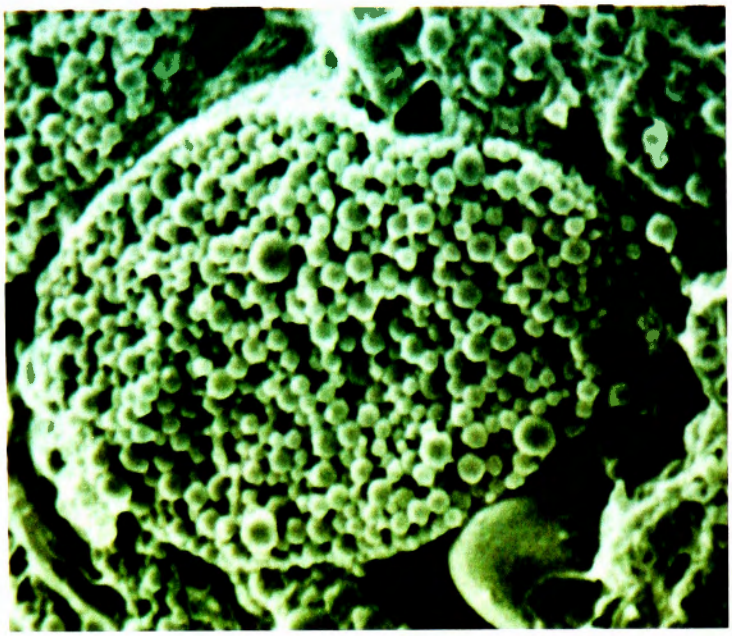

Fig. 5. SEM of a cryofractured phagocyte in the rat spleen (same sample as in Fig. 3). Numerous fat particles remain undigested. (× 10000)

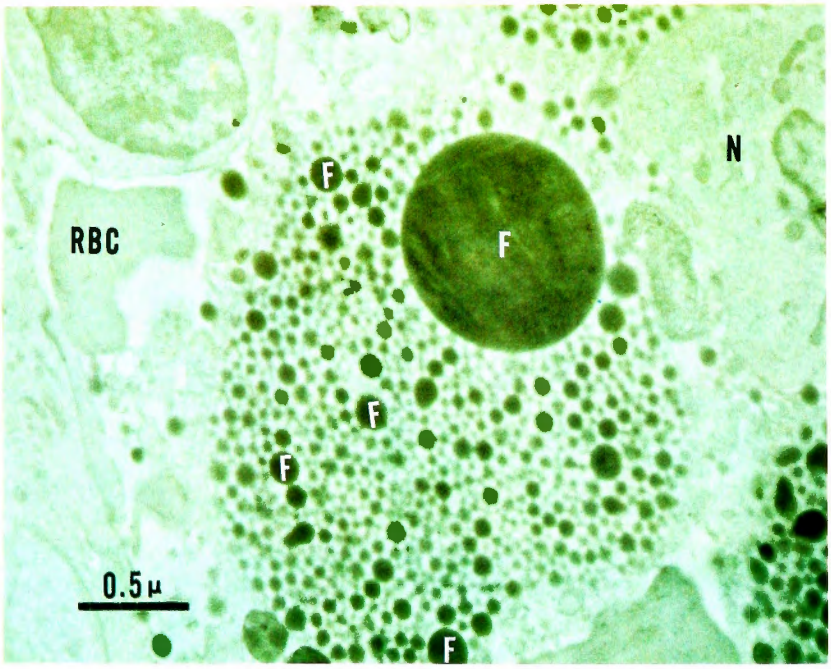

Fig. 6. TEM of a phagocyte in the rat spleen (same sample). This sample was not double stained. Fat particles have a high electron density. Compare to the that of RBC.

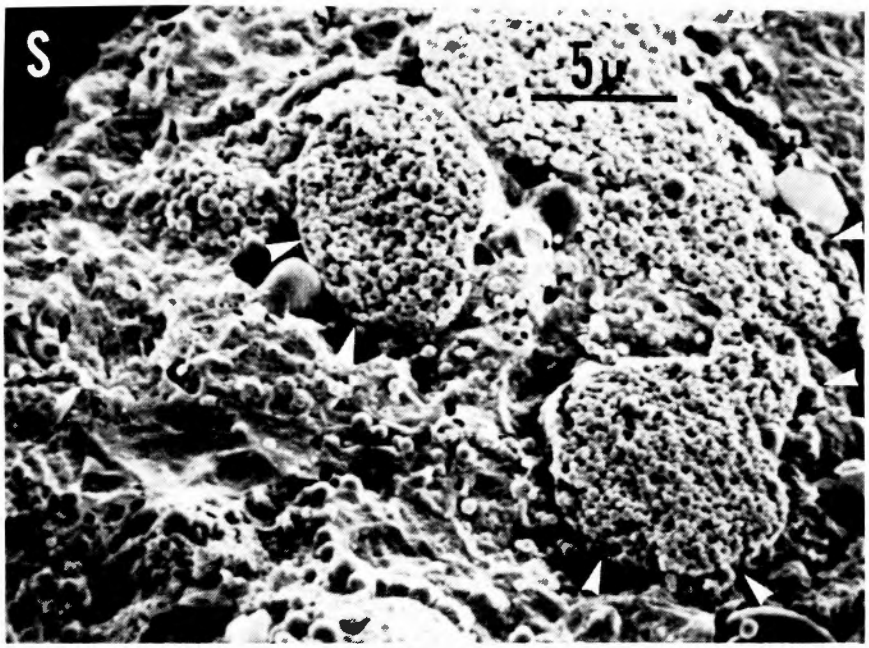

Fig. 7.

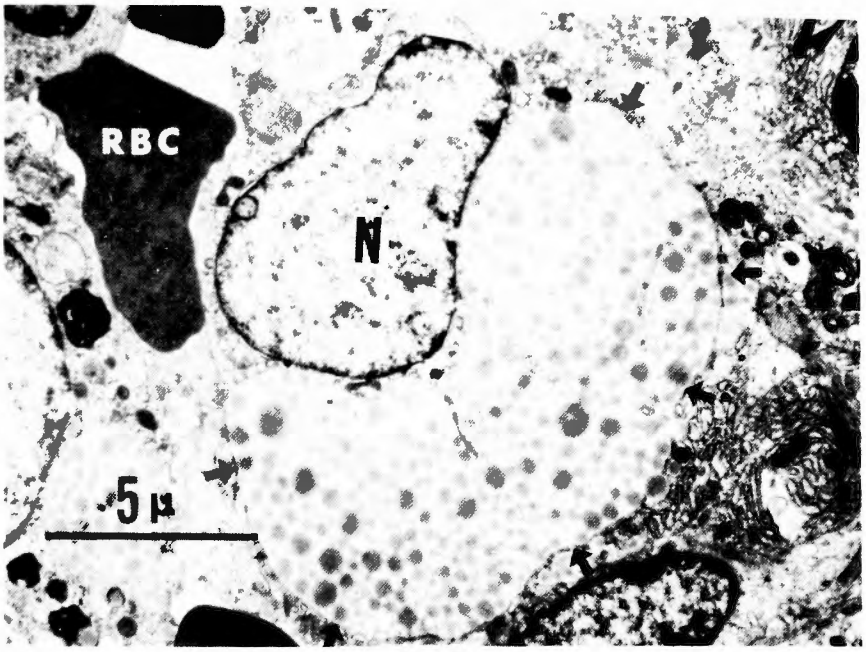

Fig. 8.

Fig. 7., 8. SEM and TEM of a phagocyte of the spleen in Case 2. Fat particles are surrounded by arrows.

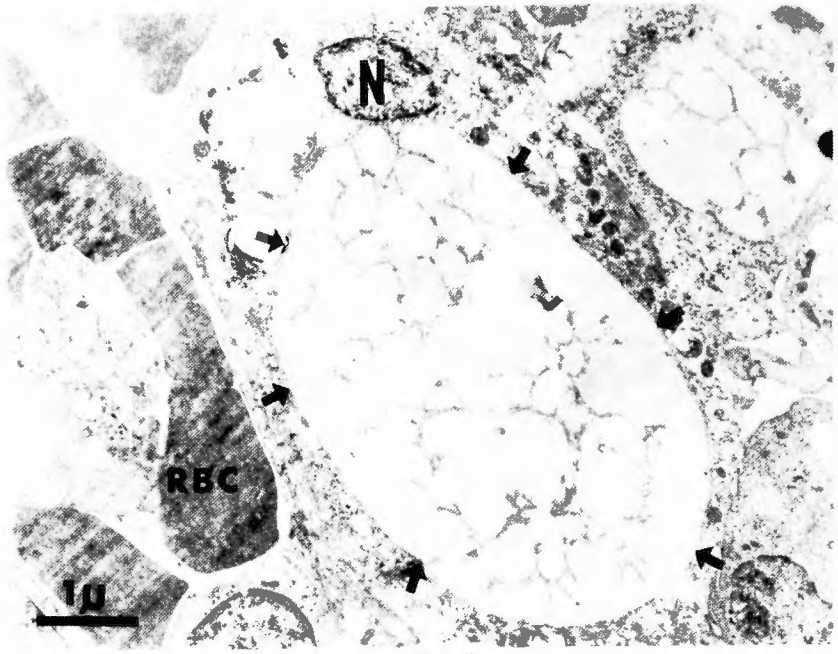

Fig. 9.

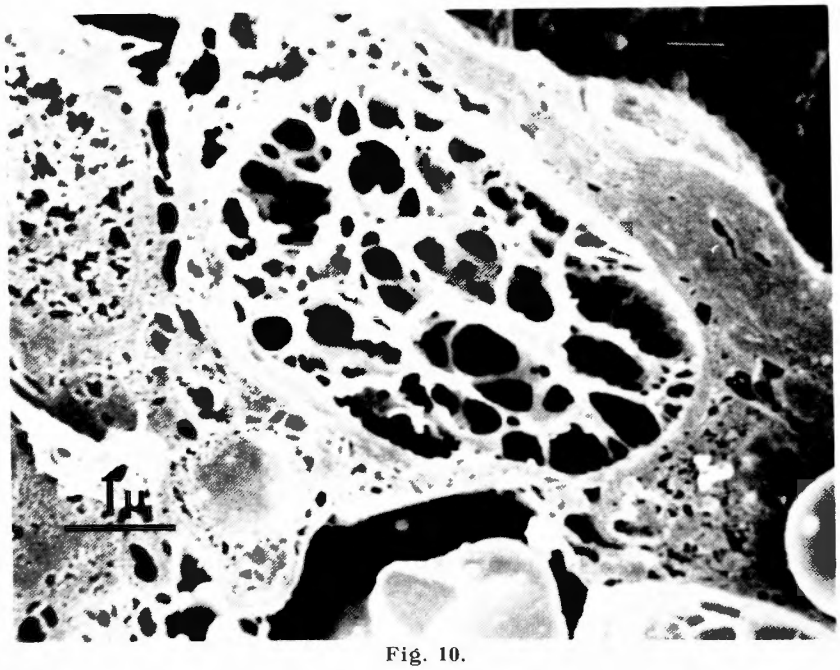

Fig. 10.

Fig. 9., 10. SEM and TEM of a phagocyte of the same sample as in Figs. 7. and 8. As malachite green was not used, the trapped fat particles are not seen. There remain only a network-like structure.
Arrows show a vesicle that contains fat particles.

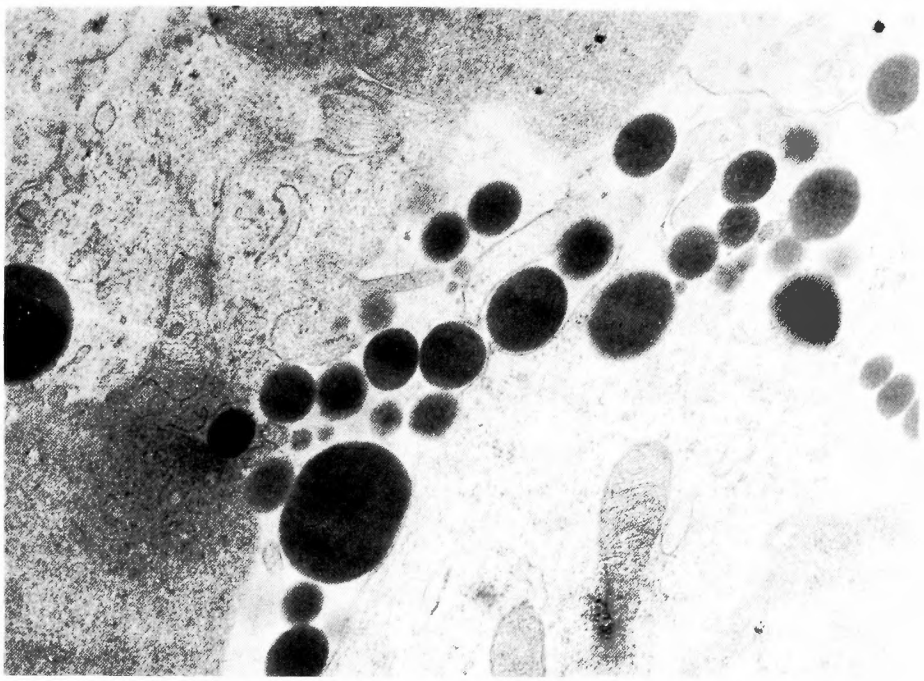

Fig. 11. TEM of the human spleen (splenic cord). Without double stain. Free fat particles were observed clearly. (×10000)

By TEM and SEM observations, significant findings compared to the control were not noted except for the intravenous fat pigments.

2. Results of the Clinical Study

Case 1.; There was no sudanophilic material in the cells of the spleen. Even by the two different perfusion fixations, no characteristic findings were observed ultrastructurally.

Cases 2 and 3; By Sudan III staining, numerous macrophages in the spleen contained large sudanophilic materials. As in the animal group, these sudanophilic globules consisted of small particles. By HE stain these globules were observed to be as large as vacuoles. The TEM and SEM findings were similar to those in animals (A) (Figs. 7, 8, 9 and 10). However, several additional findings were noted. By TEM observation with malachite green, free particles in the sinusoid of the spleen were frequently observed (Fig. 11). Rod cells of the sinusoid also phagocytized the particles and granulocytes also phagocytized the particles. Trapping of red blood cells[16] which is thought to cause a great change in the membrane by fat emulsion was unclear. By SEM observation with malachite green, free particles were also noted, and echinocytic red blood cells attached with the particles were observed in the sinusoid of the spleen or in the splenic cord (Fig. 12). Considering that numerous undigested fat particles remaining in the cytoplasm of RES in the spleen, sinusoid and splenic cord for a long time, the spleen seemed to be like a reservoir organ of intravenous fat emulsion.

Cases 4 and 5; Sudanophilic materials in macrophages in the spleen were observed only sparsely. However, intravenous fat pigments were observed in other macrophages without any sudano-

Fig. 12. SEM of the spleen as in Fig. 7. This is a figure of the splenic cord. Note free fat particles. Some of them are attached to RBC. (×10000)

philic material. By TEM and SEM observations, no characteristic findings were observed except for intravenous fat pigments (Fig. 13). It was impossible to identify intravenous fat pigments by SEM.

Discussion

The role of the spleen is still vague. Until now total splenectomy has been readily performed in trauma. However, post-splenectomy syndrome has been reported among physicians. This syndrome was thought to be a great problem among infants, but this problem is gradually being recognized as relatively common even among adults. Efforts have been made to leave the healthy part of the spleen intact in trauma. The importance of the spleen against infection specially in pneumonia has been recognized.

Forbs[4] et al recently reported necrosis of the spleen at autopsy in patients who received infusions of the intravenous fat emulsion. This finding that drugs can induce chemical splenectomy is of importance to critically ill patients who receive the intravenous fat emulsion because their ability to digest emulsified fat particles is weak. Among these patients the possibility of lipidosis[9,10] or the accumulation of the infused fat emulsion[1,4] is very high. Thus, if these patients receive the intravenous fat emulsion, a drug induced splenectomy might occur and be as a trigger of severe infection. If it were so, the decrement of RES activity might be possible[2,5,6,8,11,12,13,14,19,21,33].

From the beginning of the development of the fat emulsion, the effect of it on the spleen had been studied and concluded that first trapping of fat particles was performed in RES mainly in the liver and spleen, and the deposition of fat particles in the RES of the spleen was transient only remaining intravenous fat pigments. However, as these studies were done at the light micro-

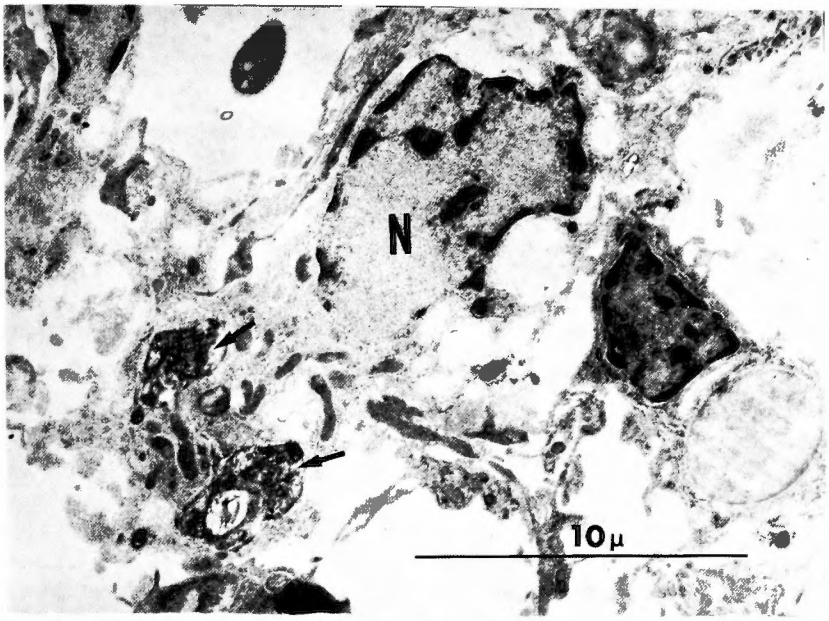

Fig. 13. TEM of the human spleen in Case 4. Arrows show intravenous fat pigment.

scopical level, the detail morphology in the RES cells was not clarified. Moreover, as these studies were performed using healthy experimental animals, the evaluation of infused patients who are critical ill has still been obscure. The possibility that the intravenous fat emulsion gives some adverse effects if it is infused to patients poor in clinical condition has been still remaining, although the fat emulsion is thought to be a safe material to those who are essentially healthy.

From these considerations, the author[22,23,24] examined morphologically the spleens infused with the intravenous fat emulsion chiefly from ultrastructural investigation. Though it has been difficult to fix and observe fat materials for specimens of TEM and SEM, the authors were able to fix tightly fat particles of fat emulsions and to make it unsoluble to alcohol dehydration using malachite green[26] in specimens for TEM and SEM. Fat particles of the emulsion were given strong electron density and they were observed as uniform black globules by TEM and as spherical particles with a smooth surface by SEM.

The particles which appeared in the macrophages and in the splenic cord in a free form were those of the fat emulsion. During short time infusion of the fat emulsion, the RES responds continuously to phagocyte fat particles. Moreover, fat particles were retained in the spleen in the free form for a long time. The spleen appeared to be reservoir of the fat emulsion. On the other hand, when the fat emulsion was infused by the one-pack method, the spleen appeared to be able to tolerate the fat emulsion comparable to a level that of short time infusion.

In this investigation, the results obtained between animal and human spleens were in good agreement.

At present, the intravenous fat emulsion has been used clinically as a safe infusate and it is an important source of the essential fatty acids as well as high calories. However, several

adverse effects have been reported. One of the reasons for these adverse effects is thought to be the method of infusion. The generally recommended way to infuse the intravenous fat emulsion is to infuse it via the peripheral vein over 2 hours. This disadvantage was reported by the authors. According to this method large doses of fat particles were retained in the spleen. The digestion and the metabolism of fat particles can proceed smoothly in patients with the remarkable tolerance. However, in critically ill patients whose macrophagic digestion of fat particles in the spleen may be weak, severe lipidosis and necrosis of the spleen can occur.

However, those who require the intravenous fat emulsion as a source of the essential fatty acids and calories are chiefly critically ill patients. Therefore, careful attention must be paid to infusing the fat emulsion. From the author's investigation the one-pack TPN is considered to be tolerable even to critically ill patients and is safer than the infusion method generally recommended.

Conclusion

1. The generally recommended method of the infusion of the intravenous fat emulsion may damage the spleen especially in critically ill patients.
2. Careful attention must be paid to the critically ill, whose ability to digest fat emulsion is weak, because the intravenous fat emulsion is a basic requirement of those who need TPN.
3. The one-pack infusion method is considered to be tolerable and safe even to the patients who are critically ill.

Acknowlegement

The author express deep gratitude to Prof. YORINORI HIKASA and Dr. HIROSHI TANIMURA for their kind advice and supervision.

This work was supported by Grand-in-aids No. 244050 for Scientific Research of the Ministry of Education, Science and Culture in 1978.

The results were presented at the 13th and 14th Annual Meeting of Clinical Electron Microscopic Association in 1980 and 1981.

References

1) Belin RP, Bivius BA, et al: Overload with a 10% soybean oil emulsion. Arch. Surg. **111**: 1391–1393, 1976.
2) Bradfield JWB: Intralipid and reticuloendothelial blockade. Lancet **22**, 1980.
3) Clark DE: Management of the injured spleen without splenectomy. J. Maine Med. Assoc. **71**: 271–273, 1980.
4) Granemark PI, Petevson LS, et al: Microcirculatory effects of emulsified fat infusions. Circ. Res. **15**: 124, 1964.
5) Fischer GW. Diminished bacterial defence with Intralipid. Lancet Oct. **18**, 1980.
6) Fischer GW: Inhibitory effect of Intralipid on reticuloendothelial function and neutrophil bactericidal activity. Pediat. Res. **13**: 494, 1979.
7) Foebs GB: Splenic lipidosis after administration of intravenous fat emulsions. J. Clin. Path. **31**: 765–771, 1978.
8) Freise J, Müller WH: Uptake of liposomes and sheep red blood cells by the liver and spleen of rats with normal or decreased function of the reticuloendothelial system. Res. Exp. Med. **178**: 263–269, 1981.

9) Frund U, Gilbert RJ et al: Iatrogenic lipidosis following prolonged intravenous hyperalimentation. Am. J. Clin. Nutr. **28**: 1156–1160, 1975.
10) Hesson IB, Walton J, et al: Postmortem finding in three patients treated with intravenous fat emulsions. Arch. Surg. **114**: 66–68, 1979
11) Jarstrand LO, Bergkem L, et al: Human granulocyte and reticuloendothelial system function during intralipid infusion. JPEN **2**: 663–670, 1978.
12) Fischer GW: Intralipid and reticuloendothelial clearance Lancet Dec. **13**, 1980.
13) Kim W, Pfeifer J, et al: Measurement of phagocytic activity of reticuloendothelial system by Intralipid. Surg. Forum **28**: 85–87, 1977.
14) Kolski GB: Soybean oil emulsion induces a selective and reversible inhibition of C2 production by human mononuclear phagocytes. J. Immunol. **126**: 2267–2271, 1981.
15) Leonard AS, Giebink GS, et al: The overwhelming postsplenectomy sepsis problem. World J. Surg. **4**: 423–427, 1980.
16) Levesque MJ, Groom AC: A comparative study of the sequestration of "abnormal" red cells by the spleen. Can. J. Physiol. Pharmacol. **58**: 1317–1325, 1980.
17) Littleton HR: Overwhelming postsplenectomy infection syndrome: Report of case and review of the literature. JAOA **80**: 624–627, 1981.
18) Lowdon AGR: Risk of serious infection after splenectomy. Brit. Med. J. **1**: 446, 1966.
19) Luzio D, Blickens DA: Influence of intravenously administered lipids on reticuloendothelial function. J. Reticulo- endothel. Soc. **3**: 250–270, 1966.
20) Macpherson AIS: Prognosis after splenectomy. A review of 322 cases. J. R. Coll. Surg. Edinb. **26**: 12–17, 1981.
21) Melly MA: Microbial growth in lipid emulsions used in parenteral nutrition. Arch Surg. **110**: 1479–1481, 1975.
22) Miki K, Tanimura H, et al: Electron microscopical study on the metabolism of intravenous fat emulsion. J. Clin. Electron Microscopy. **13**: 755–756, 1980.
23) Miki K, Tanimura H, et al: A morphological study on the metabolism of intravenously infused fat emulsion, especially in the spleen. Jap. J. Surg. Metabolism and Nutr. **14**: 136, 1980.
24) Miki K, Tanimura H, et al: The effects of two infusion methods of intravenous fat emulsion on the fat metabolism in the liver and spleen. J. Clin. Electron. Microscopy **14**: 749, 1981.
25) Oakes DD, Charsers C: Changing concepts in the management of splenic trauma. Surg. Gynecol. Obstet. **153**: 181–185, 1981.
26) Pourcho RG: Malachite green applications in electron microscopy. Stain Technol. **53**: 29–35, 1978.
27) Kevin C: Fulminant pneumococcal septicaemia in an adult following splenectomy. NZ Med. J. **89**: 85–86, 1979.
28) Robinette CD: Splenectomy and subsequent mortality in veterans of the 1939–45 war. Lancet **2**: 127–129, 1977.
29) Sherman R: Rationale for and methods of splenic preservation following trauma. Surg. Clin. North Am. **61**: 127–134, 1981.
30) Sherman R: Perspective in management of trauma to the spleen. 1979 Presidential Address, American Association for the surgery of trauma. J. Trauma **20**: 1–13, 1980.
31) Strauch GO: Preservation of splenic function in adults and children with infused spleens. Am. J. Surg. **134**: 478–183, 1979.
32) Sullivan JL, et al: Immunune response after splenectomy. Lancet **1**: 178–181, 1978.
33) Thompson SW, Jones LD, et al: Testing of fat emulsions for toxicity III. toxicity studies with new fat emulsions and emulsion components. Am. J Clin. Nutr. **16**: 43–61, 1965.
34) Toit DF, Villet WT, et al: Fat emulsion deposition in mononuclear phagocyte system. Lancet **1**: 898, 1978.

和文抄録

新しい固定法による静注用脂肪乳剤の超微形態

（Ⅱ） 脂肪乳剤の短時間投与法とワンパック方式投与法における脾臓に対する影響について

京都大学医学部外科学教室第2講座（指導：日笠頼則教授）
三 木 毅一郎

静注用脂肪乳剤は，効率のよいカロリー源であり，又唯一の脂肪の非経口的供給源として重要な輸液剤である．ところが，最近，脂肪乳剤投与を受けた患者の剖検例で脾臓の脂肪乳剤蓄積による壊死が報告されたことなどから，脾臓内での乳剤粒子の示す態度を形態学的に検討した．

摘出4人脾並びにラット脾を灌流固定し，TEM·SEMにより観察した．灌流固定液としてはマラカイトグリーン加グルタールアルデヒド液を用いたが，それにより乳剤粒子と組織とを同時に固定することができた．

その結果，短時間に脂肪乳剤を投与すると脾臓の網内系は数時間乳剤粒子の過食状態に陥り，乳剤粒子は長時間にわたり未消化のままそこに残存・停滞する．このことは，免疫能の低下，感染誘発などの可能性を示唆した．更に脂肪乳剤の蓄積の可能性も示唆していた．

逆に，one-pack 方式による8時はこのような所見はみられず，それが脾臓に対して負担の軽い投与法と考えられた．

前述の赤血球に関する研究成果と併せ考え，one-pack 方式による投与は脂肪乳剤投与の生体に対する悪影響を最少限に止める最も生理的な投与方法であると考えられ，特に重症例ではそうすることが是非共必要と思われる．